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ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *POLYALTHIA LONGIFOLIA* (MEPL) IN EXPERIMENTALLY INDUCED PAIN IN MICE

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ABSTRACT

Objective: The main objective of the present study was to evaluate the analgesic activity of methanolic extract of *Polyalthia longifolia* (MEPL) in swiss albino mice by using Eddy's hot plate and Acetic Acid Writhing Test. **Methods:** Analgesic activity of *MEPL* was studied using Eddy's hot plate at temperature 55˚C±0.5˚C and acetic acid (4 g/kg p.o.) induced writhing test in mice. Pentazocin (10 ml/kg b.w. i.p.) and Aspirin (100mg/kg. b.w. p.o.,) was used as a standard reference respectively in this study. In Eddy's hot plate method, the latency was recorded before and after 15, 30, 60 and 120 min following oral administration of 100 & 300 mg/kg of extract to different groups of six animals each. In acetic acid writhing test, the mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:- (Control mean - Treated mean/ Control mean) ×100 %. **Results & Conclusion:** In Eddy's hot plate**,** Pentazocin alone (10ml/kg) treated mice showed significant ($p<0.001$) increased in time when compared with normal control animals. Treatment with MEPL 100 ($p<0.01$), 300mg/kg ($p<0.001$) to normal control animals showed significant increase in the mean latency time.In acetic acid induced writhing test, Aspirin (100mg/kg) treated mice showed significant ($p<0.001$) decrease in writhings when compared with acetic acid control animals. Treatment with MEPL 100 ($p<0.01$), 300mg/kg ($p<0.001$) to normal control animals showed significant decrease in the writhing, Thus it was interesting to see that phenolic compounds present in plant exhibits potent analgesic actions. Thus from the present investigation it can be said that extract at doses 100 & 300 mg/kg, p.o. widely used acute anlagesic model for studying analgesic agent and was found to be statistically significant widely.

INTRODUCTION:

Pain is an unpleasant feeling often caused by intense or damaging stimuli, such as stubbing a toe, burning a finger, putting alcohol on a cut, and bumping the funny bone. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1]. Pain is the most common symptom of injury and disease, and descriptions can range in intensity from a mere ache to unbearable agony. Nociceptors have the ability to convey information to the brain that indicates the location, nature, and intensity of the pain [2]. Herbs have

recently attracted attention as health beneficial foods and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases including inflammation, pain, liver disease, ischemia, reperfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer with relatively little knowledge regarding their modes of action. *Polyalthia longifolia* Benth and Hook (Annonaceae) is a large genus of shrubs and tree which is distributed in tropics and subtropics regions of India, Pakistan and Sri Lanka [3,4]. It has been shown to possess variety of medicinal properties in indigenous systems of medicine. Almost all parts of the plants are used in the traditional system of medicine for the treatment of various ailments in human beings. Till date, more than 20 active principles have been isolated and characterized from the *P. longifolia* in aqueous, methanolic, and ethanolic and chloroform extracts [5]. Extracts in different solvents from various parts of *P. longifolia* (e.g. leaves, root bark, stem bark, green berries, flowers etc.) demonstrated anti-bacterial and anti-mycotic properties [6]. Alcoholic extracts of the plant has also been shown to exhibit antiulcer activity in animal models [7,8]. Recently study have demonstrated that the stem bark extract of *P. longifolia* (Sonn) Thw. var. pendula had displayed potent antiplasmodial activity thatstrongly supports its use in phytomedicines for treating malaria [9]. *P. longifolia* has the potential to be explored as a cardioprotective medicinal plant & showed a significant ability to reduce blood pressure [10]. Bark extract of *P. longifolia* has high content of phytosteroids, which may mimic estrogen structurally and therefore may modulate the estrogen receptor expression. Though, there is no specific evidence but bark extract may act as SERM (selective estrogen receptor modulators) to effect on the downstream signaling cascades driven through estrogen receptor was demonstrated [11]. In another study, the ethanolic and aqueous extracts of *P. longifolia* were found to reduce Carrageenan induced edema and inflammation [12]. Hepatoprotective potential of plant has also been demonstrated in an animal study [13,14]. Literature reviews indicated that there is no scientific report on analgesic property of the title plant till date. Hence, the presence investigation is aimed to assess protective role of *Polyalthia longifolia leaves* extract against Eddy's hot plate and acetic acid writhing test.

MATERIALS AND METHOD:

Collection of plant material:

Plant material was collected from Sri Venkateshwara University Campus, Tirupati, chittoor (dist), India. The plant was identified and authenticated by Dr K Madhava Chetty, Assisstant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, India. The herbarium was prepared and kept in college.

METHODOLOGY:

Methanolic extract of *polyalthia longifolia* (MEPL):

The whole plant was washed with distilled water and shade dried for two weeks [15]. After drying, the dried plant material was powdered with mechanical grinder and passes the powder with sieve no. 22 to get uniform particle size. The powder is packed in Soxhlet apparatus for defatting with n-hexane for 3 days. The plant powder (marc) was air dried after defatting. Again it is packed in Soxhlet apparatus for methanol extraction for 18hrs until to get clear solution in syphon tube.

Experimental animals:

Swiss albino mice were obtained from Sri Venkateshwara Enterprises, Bangalore (Karnataka) for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12 h

light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental, control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free assessed to standard pellets diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Vasavi Institute of Pharmaceutical Sciences. (**Ref: VIPS/IAEC.CLEAR/106/11-12**).

Preparation of dose:

Weighed quantity of Methanolic extract of *Polyalthia longifolia* (MEPL) was suspended in water using 12% tween 80 and administered orally to experimental animals. Suspension of MEPL was prepared freshly. The MEPL was administered at doses of 100 mg/kg (L.D) and 300 mg/kg (H.D) for each animal as per previous study.The experiments were conducted before & after the oral administration.

Assessment of analgesic activity

Eddy's hot plate method [16-18]: Six animals in each group weighing about 20-25g. **Treatment schedule** - Animals were randomly divided equally into four groups of six animals each.

Group 1: (Normal Control) received 12% Tween 80,1ml/kg orally
Group 2: **(**Standard) received Pentazocin (10 mg/kg i.p)
Group 3: (Low dose) received MEPL (100 mg/kg p.o.)
Group 4: (High dose) received MEPL (300 mg/kg p.o.)

The latency was recorded before and after 15, 30, 60 and 120 min following oral administration of 100 & 300 mg/kg of extract to different groups of six animals each. Average reaction times were then calculated and the percentage variation calculated using the following ratio:

$$\text{percentageprotection} = \frac{\text{druglatency} - \text{baselinelatency}}{\text{baselinelatency}} \times 100$$

Acetic acid-induced writhing tests [19-21]: Six animals in each group weighing about 20-25g.

Treatment schedule: Swiss albino mice were divided into five groups (n = 6).

Group I: Received 12% tween 80 1ml/kg and writhing reflexes noted for period of 15min
Group II: Received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes.
Group III: Received aspirin (100 mg/kg b.w., p.o.) and acetic acid (1% v/v, 10 ml/kg b.w., i.p.)
Group IV: Received MEPL (100 mg/kg b.w.,p.o.) and acetic acid (1% v/v, 10 ml/kg b.w., i.p.)
Group V: Received MEPL (300 mg/kg b.w.,p.o.) and acetic acid (1% v/v, 10 ml/kg b.w., i.p.) Thirty minutes after aspirin and extract administration, group II to V received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min. The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:- (Control mean - Treated mean/ Control mean) ×100 %.

Statistical analysis: The data are represented as mean ± standard error of mean (SEM). Degree of significance was assessed by Anova

RESULTS:

Eddy's hot plate: The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of a electrically heated surface.

Table 1: Analgesic Activity of MEPL by Eddy's hot plate

Group	Dose	Mean reaction time (in sec) by hot plate method				
		0min	15min	30min	60min	120min
Group I (control)	1ml/kg	3.0 ± 0.06	3.0 ± 0.14	3.0 ± 0.01	3.0 ± 0.01	3.0 ± 0.17
Group II (standard)	10ml/kg	3.0 ± 0.12***a	6.0 ± 0.14***a	10.0 ± 0.14***a	14 ±0.16***a	17 ± 0.12***a
Group III (Low dose)	100mg/kg	2.8 ± 0.11***b	4.0 ± 0.10***b	6.0 ± 0.10***b	10 ± 0.09***b	14 ± 0.12***b
Group IV (high dose)	300mg/kg	3.0 ± 0.12***b	5.0 ± 0.08***b	8.0 ± 0.08***b	12 ± 0.08***b	16 ± 0.03***b

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Dunnet's test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001,compared to positive test. [a] Pentazocin increased mean reduction time was significantly different from Normal control group. [b] Treated group were significantly different from Pentazocin increased mean reduction time.

Table 2. Analgesic effect of MEPL on acetic acid induced writhing in mice.

Group	Dose	Number of writhes	% of protection
Control group	10ml/kg	19.85±1.872	----------
Acetic acid	10ml/kg	52.83 ±1.400	----------
Acetic acid+ Aspirin	100mg/kg	17.26 ±1.606***a	67.32
Acetic acid + Low dose	100mg/kg	21.31 ±1.661***b	59.66
Acetic acid + High dose	300mg/kg	18.16±1.291***b	65.63

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Dunnet test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001,compared to positive test. [a] Aspirin decreased number of writhes was significantly different from Normal control group. [b] Treated group were significantly different from Aspirin which decreased number of writhes

The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. MEPL when given in doses of 100 & 300 mg/kg, p.o. elicited a significant analgesic activity in the hot plate as evidenced by increase in latency time in seconds (Table 1) as compared with vehicle control at the end of 30 min. The increase in latency time was dose dependant. The analgesic activity data (Hot plate method) are presented in Table 1. Latency time was noted at 0, 15, 30, 60 and 120 min after the administration of vehicle, standard and plant extract. Pentazocin alone (10ml/kg) treated mice showed significant (p<0.001) increased in time when compared with normal control animals. Treatment with MEPL 100 (p<0.01), 300mg/kg (p<0.001) to normal control animals showed significant increase in the mean latency time (Table no:1)

Acetic acid induced writhing test:

The chemical agents can produce nociceptive reactions in mice. Intra-peritoneal injection of phenyl para quinone, bradykinin or dilute acetic acid

(1-3% v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain.
Aspirin (100mg/kg) treated mice showed significant ($p<0.001$) decrease in writhings when compared with acetic acid control animals. Treatment with MEPL 100 ($p<0.01$), 300mg/kg ($p<0.001$) to normal control animals showed significant decrease in the writhing (Table no:2)

DISCUSSION & CONCLUSION:

Acute pain is mediated through nociceptors that fire in response to chemicals released during tissue damage, including leukotrienes, bradykinins, serotonin, histamine and thromboxanes [11]. The significant increase in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems [22]. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

Eddy's Hot Plate method:

The hot plate test was selected to investigate central antinociceptive activity because it has several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibit prostaglandin synthesis [23]. The MEPL (100 mg/kg, p.o.) show significant ($P > 0.05$) increase in the mean basal reaction time in hot plate method compared to control. The MEPL at dose (300 mg/kg, p.o.) also showed significant ($P < 0.05$) increase in the mean basal reaction time. Analgesic activity was comparable with the standard drug pentazocin. As phytochemical tests showed presence of phenolic compounds in methanolic extract of *Polyalthia longifolia* , they might supress the formation of prostaglandin and bradykinins.

Acetic acid writhing test:

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response. The effect of the extract against noxious stimulus may be an indication that depressed the production of irritants and thereby reduction in number of writhes produced by animals. The writhing induced by chemical substance is due to sensitisation of nociceptors by prostaglandins. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting nociceptives [24]. This response is thought to involve peripheral local peritoneal receptors. This result indicates that analgesic effect of methanolic extract of *Polyalthia longifolia* might be mediated by inhibiting synthesis or action of prostaglandins. Aspirin (100mg/kg) treated mice showed significant ($p<0.001$) decrease in writhings when compared with acetic acid control animals. Treatment with MEPL 100 ($p<0.01$), 300mg/kg ($p<0.001$) to normal control animals showed significant decrease in the writhing. Thus it was interesting to see that phenolic compounds present in plant exhibits potent analgesic actions [25]. Thus from the present investigation it can be said that extract at doses 100 & 300 mg/kg, p.o. widely used acute anlagesic model for studying analgesic agent and was found to be statistically significant widely and was found to be statistically significant ($p<0.005$) antinociceptive. In conclusion, the results of the present study indicated that extract of plant used possess might contain constituents capable of relieving or

modifying responses to pain caused by either thermal or chemical stimulation of the noiceptors mediated by both central and peripheral mechanisms. This could provide a rationale for the use of these plants in pain disorders in folk medicine. These reports may serve as a foot step in the research of potent Analgesic drug.

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